

REMARKS

Applicants respectfully request entry of the foregoing and continued examination of the subject matter identified in caption, as amended, pursuant to and consistent with 37 C.F.R. § 1.114, and in light of the remarks which follow.

As noted in the Office Action of October 6, 2003, claims 18-35 are pending. Claims 19-35 are amended herein to address simple issues of format and grammar. Independent claim 18 is amended herein to recite that the DNA extraction step excludes a DNA precipitation step. Basis for this amendment may be found throughout the specification and claims as-filed, especially at least at page 33, lines 1-12 and in Protocol 2, on page 36, lines 5-13.

New claims 36 to 45 are presented herein. Basis for these new claims may be found throughout the specification and claims as-filed, especially at least at page 44, line 19 to page 45, line 23; page 45, lines 24-30; page 46, lines 14-22; page 58; page 59, line 20 to page 60, line 26; page 60, line 27 to page 62, line 8; page 61, lines 21-31; pages 65-67; and page 68, lines 26-30.

Thus, no prohibited new matter is believed to have been presented by way of the present Amendment. Any subject matter canceled by way of this Amendment has been canceled without prejudice to or disclaimer of that subject matter. Applicants reserve the right to file at least one continuation application directed to any subject matter canceled by way of the present Amendment.

Finally, Applicants respectfully request that the U.S. Patent and Trademark Office use the new attorney docket number for this case be used as reference, Attorney Docket No. 034217-003.

Priority

This application claims priority under 35 U.S.C. § 120 to U.S. Patent Application No. 08/667,493, filed June 24, 1996 and U.S. Patent Application No. 08/311,553, filed September 23, 1994. The first sentence of the specification is amended herein to recite the required priority information, as previously submitted in Applicants' Amendment of April 6, 2004.

Specification

The Examiner notes that on page 22, line 7, the specification recites "cite?". The specification is amended herein to remove this term, as previously submitted in Applicants' Amendment of April 6, 2004. Thus, this objection is obviated.

Claim Rejections Under 35 U.S.C. § 102

Claims 18-32, and 35, stand rejected under 35 U.S.C. § 102(b) as being anticipated by Meltzer *et al.* (PNAS, Vol. 88, pages 4976-4980, 1991).

Meltzer *et al.* is cited for disclosing a method of detecting loss of heterozygosity (LOH) using PCR. Meltzer purportedly discloses a genotyping method comprising obtaining a specimen of cells containing DNA, inspecting the cells to determine target cells, choosing target cells based on morphological features characteristic of a disease and separating the target cells. Meltzer purportedly then discloses that the tumor cells were microdissected to separate them from adjacent normal cells to achieve an enrichment of about 70% or greater. Meltzer then purportedly places the separated piece into a container, centrifuging to make a pellet and withdrawing supernatant to obtain DNA by placing the separated tissue into a container, grinding it into a powder, incubating it in a container in a lysis solution

containing proteinase K (0.5 mg/ml), and treating it with phenol/chloroform and ethanol. Applicants respectfully traverse.

Applicants submit that Meltzer fails to recite every element of the presently claimed invention. To anticipate a claim, a single prior art reference must teach each and every element of the claimed invention. *See M.P.E.P. § 2131; Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631, 2 U.S.P.Q.2d 1051, 1053 (Fed. Cir. 1987); *Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1379, 231 U.S.P.Q. 81, 90 (Fed. Cir. 1986).

Meltzer discloses a method of DNA extraction of grinding of tissues with a mortar and pestle, and then incubating the ground tissues in a lytic solution overnight. DNA is then precipitated overnight at -20 degrees Celcius after the addition of NaCl and ethanol. Finally, the DNA was washed with ethanol (as described by Meltzer at page 4976).

The present invention is directed to a method of topographic genotyping, wherein the DNA extraction step does not include an ethanol precipitation step. (See, for example, specification, pages 32 and 36). Meltzer creates a DNA pellet, dries the pellet using ethanol evaporation, and then rehydrates the pellet). This avoidance of the DNA precipitation step in DNA extraction is a great time and cost saving benefit of the present invention, as precipitation steps involve at least overnight incubation and often many other steps. Meltzer states that during DNA extraction, DNA was precipitated overnight at -20 degrees Celcius after the addition of 0.02 volume of 5 M NaCl and 2.5 volume of 100 percent ethanol. Thus, Meltzer recites the additional step of DNA ethanol precipitation, which is not a claimed step and is specifically excluded.

To clarify the presently claimed invention, independent claim 18 is amended herein to recite that the step reciting the extraction of the DNA from the target does not include a precipitation step.

Thus, Meltzer fails to recite all of the elements of the present invention as amended herein. Applicants respectfully request that the rejection under 35 U.S.C. §102 be withdrawn.

Claim Rejections Under 35 U.S.C. § 103

Claim 33 stands rejected under 35 U.S.C. § 103(a) as purportedly unpatentable over Meltzer *et al.* (PNAS, Vol. 88, pages 4976-4980, 1991) in view of Erlich (*PCR Technology*, 1990).

As noted above, Meltzer *et al.* purportedly discloses a method of detecting LOH using PCR. The teachings of Meltzer are summarized above. The Examiner admits that Meltzer fails to disclose bringing the temperature back to 55 degrees Celcius.

Erlich is referenced as purportedly disclosing the "standard" reaction for PCR. Specifically, Erlich purportedly discloses that PCR can be performed in a DNA Thermal Cycler using the Step-Cycle program set to denature at 94 degrees (not more than 99 degrees) followed by annealing at 55 degrees for 20 seconds. The Examiner argues that it would have been obvious to the skilled artisan to have modified the PCR conditions of Meltzer, with the "standard" protocol suggested by Erlich. Applicants traverse.

In order to establish a case of *prima facie* obviousness, three basic criteria must be met: (1) there must be some suggestion or motivation to modify the reference or combine reference teachings, (2) there must be a reasonable

expectation of success, and (3) the prior art reference(s) must teach or suggest all of the claim limitations. See M.P.E.P. 2142. Applicants respectfully submit that these criteria have not been met in the present Office Action.

The cited references, alone or in combination, fail to recite all of the elements of the presently claimed invention or to provide an expectation of success or motivation to arrive at the claimed invention. As noted above, the present invention is directed to methods of typographic genotyping, with many unexpected benefits. For example, the present invention allows for effective extraction from fixed, embedded tissues without sacrificing the quality of results, despite the age of the specimen. Further, the methods may be performed on mixtures of cellular components and necrotic, crushed sites which would otherwise be too damaged to use. Multiple analysis may be done on one sample without recutting.

Furthermore, it is beneficial to directly proceed to nucleic acid amplification following DNA extraction without a DNA precipitation step, as taught by Meltzer. The removal of a DNA precipitation step saves time, money and allows for minimal handling of the tissue. This direct use of the tissue in the amplification step, rather than precipitating DNA out of the sample first, is possible because of the new understanding in the present invention that DNA from tissue is required only for the initial amplification cycles of PCR, in order to generate sufficient amounts of DNA for further cycles of amplification. In fact, it has been discovered that the tissue sample need only be optimally treated as indicated in the present invention to allow sufficient DNA to be available for initial amplification. By properly preparing the sample for direct use in the amplification step, the entire step of DNA precipitation (as taught by Meltzer) can be avoided, resulting in a tremendous saving of time and money.

The cited references, alone or in combination, fail to recite a method of topographic genotyping without a precipitation step required for DNA extraction prior to amplification. Meltzer, the primary reference, clearly requires an ethanol precipitation step in order to extract DNA from the samples. Meltzer also discloses running test electrophoresis gels on the extracted DNA to confirm that it was intact and of appropriate concentrations. Only once these steps were completed was the following PCR amplification step attempted.

Thus, Meltzer fails to motivate the skilled artisan to attempt the methods disclosed without a precipitation step. As Meltzer also emphasizes the purity of the extracted DNA pellet obtained from a precipitation step, there would be no expectation of success by the skilled artisan with the claimed invention.

The secondary reference, Erlich, in combination with Meltzer, fails to remedy these deficiencies, and does not provide the missing motivation or expectation of success. Erlich merely discloses that PCR may take place at a temperature no greater than 99 degrees Celcius, and is cited by the Examiner to remedy deficiencies in Meltzer with regard to PCR procedure only. Thus, upon combination with Meltzer, Erlich fails to recite the elements of the presently claimed invention, to provide the skilled artisan with motivation to modify the cited references to arrive at the present invention, and finally fails to provide the skilled artisan with an expectation of success with regard to the present invention. In light of the above, Applicants request that the rejections under 35 U.S.C. § 103 be withdrawn.

Claim 34 stands rejected under 35 U.S.C. § 103(a) as purportedly unpatentable over Meltzer *et al.* (*PNAS*, Vol. 88, pages 4976-4980, 1991) in view of Erlich (*PCR Technology*, 1990), and in further view of Teramoto *et al.* (*Jpn. J. Cancer Res.* 83:329-333 (1992)).

Meltzer *et al.* is cited as above. The Examiner notes that Meltzer does not specifically disclose the use of paraffin sections which are between 2-6 μm thick. Thus, Teramoto is cited for purportedly disclosing that for PCR of DNA obtained from microscopically identified cells, 6 μm thick sections were used. The Examiner argues that it would have been obvious to the skilled artisan to have modified the paraffin sections used in the method of Meltzer with the smaller sections taught by Teramoto. Applicants respectfully traverse.

As stated above, Meltzer, in combination with Erlich, fail to recite the elements of the present invention, to provide the skilled artisan with motivation to modify the cited references to arrive at the present invention, and finally to provide the skilled artisan with an expectation of success with regard to the present invention. The Teramoto reference fails to remedy these deficiencies of Meltzer and Erlich in combination.

Teramoto is cited as a secondary reference against claim 34 only, as Teramoto purportedly recites that during the performance of PCR of DNA obtained from microscopically identified cells, 6 μm thick sections may be used. Thus, Teramoto is merely cited as further support for the PCR methods of the primary reference, Meltzer. Teramoto, in combination with the other two references, also fail to recite the elements of the present invention, to provide the skilled artisan with motivation to modify the cited references to arrive at the present invention, and finally to provide the skilled artisan with an expectation of success with regard to the present invention.

Further, Applicants respectfully submit that unexpected results are in fact present with respect to the claimed methods.

It is a well established legal precedent that the presence of an unexpected, advantageous or superior result is evidence of nonobviousness. See M.P.E.P. § 716.02(a); *In re Papesch*, 315 F.2d 381, 137 U.S.P.Q. 43 (C.C.P.A. 1963). Along these lines, it is also well established that "a greater than expected result" is evidence of nonobviousness. See M.P.E.P. § 716.02(a); *In re Corkill*, 711 F.2d 1496, 226 U.S.P.Q. 1005 (Fed. Cir. 1985).

As noted in the present specification, the rate limiting step when handling fixative treated tissue specimens for genetic analysis is effective and specific DNA amplification. DNA that has been exposed to chemical fixatives are often unsuccessfully or only poorly amplified. Previously, the investigator had to sacrifice large amounts of fixative treated tissue or abandon the use of fixative treated tissues altogether.

However, surprisingly, it is a misconception that fixative treated tissue provides an inadequate amount of starting DNA for nucleic acid amplification. In reality, the tissue obtained from 1-4 micron thick histologic sections of a small biopsy specimens, when handled properly, provides sufficient material for consistent and effective DNA amplification. In fact, when one attempts to add more fixative treated tissue to an amplification reaction, the results of the amplification are often poor. Applicants have discovered that it is important to use a small rather than a large amount of tissue to initially trigger the amplification reaction. Once triggered in the first few cycles to copy a sufficient quantity of DNA from the tissue template, the remainder of the amplification reaction goes forth in the buffer solution without significant participation of the original tissue DNA.

In light of the above, Applicants request that this rejection under 35 U.S.C. § 103 be withdrawn.

C O N C L U S I O N

From the foregoing, further and favorable action in the form of a Notice of Allowance is respectfully requested and such action is earnestly solicited.

In the event that there are any questions concerning this amendment or the application in general, the Examiner is respectfully requested to telephone the undersigned so that prosecution of the application may be expedited.

If there are any questions concerning this paper or the application in general, Applicants invite the Examiner to telephone the undersigned at the Examiner's earliest convenience.

Respectfully submitted,

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